PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

In its capacity as elected Office

Date of mailing (day/month/year) 09 May 2001 (09.05.01)	
International application No. PCT/EP00/08157	**Applicant's or agent's file reference** WOB 99 AW IDM BIOM
International filing date (day/month/year) 22 August 2000 (22.08.00)	**Priority date** (day/month/year) 30 August 1999 (30.08.99)
Applicant BARTHOLEYNS, Jacques	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 February 2001 (09.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Juan Cruz Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WOB 99 AW IDM BIOM		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08157	22/08/2000	30/08/1999
International Patent Classification (IPC) or national classification and IPC A61L27/40		
Applicant I.D.M. IMMUNO-DESIGNED MOLECULES et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/02/2001	23.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Ludwig, G Telephone No. +49 89 2399 8698

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08157

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-8 as originally filed

Claims, No.:

1-10 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 9-10.

because:

☒ the said international application, or the said claims Nos. 9-10 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-3, 6-10
	No:	Claims	1, 4-5
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10
Industrial applicability (IA)	Yes:	Claims	1-8 (9-10 - cf. separate sheets)

No: Claims

2. Citations and explanations
see separate sheet

D1: US-A-5 902 741 (DUNKELMAN NOUSHIN ET AL) 11 May 1999 (1999-05-11)

cf. the citations indicated in the international search report

Item V:

1. Document D1 discloses a threedimensional cartilage framework to form stromal tissue comprising chondrocytes/fibroblasts and, inter alia macrophages and monocytes (cf. also claims 1, 5-6, and 23 (collagen)).

   Claims 1 and 4-5 are not regarded as novel vis-a-vis document D1. Nothing inventive is seein in claims 2-3 and 6-10 having regard to this document (cf. also claim 3: "are liable to"; claims 7-8: "comprising").

2. For the assessment of the present claims 9-10 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Item III:

3. Claims 9-10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/15753 A1

(51) International Patent Classification[7]: A61L 27/40, 27/38

(21) International Application Number: PCT/EP00/08157

(22) International Filing Date: 22 August 2000 (22.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99402149.1 30 August 1999 (30.08.1999) EP

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/15753 A1

(54) Title: NEW HUMANIZED BIOMATERIALS, A PROCESS FOR THEIR PREPARATION AND THEIR APPLICATIONS

(57) Abstract: The invention relates to humanized biomaterial comprising a porous biocompatible composite material customized and implanted with monocyte derived cells preferably with macrophages.

NEW HUMANIZED BIOMATERIALS, A PROCESS FOR THEIR PREPARATION AND THEIR APPLICATIONS

The invention relates to new humanized biomaterials, a process for their preparation and their applications.

Tissue repair is needed after severe bone fractures, cartilage loss or general fragilization during ageing.

Artificial metallic or even ceramic prostheses are not very well integrated within host tissues and replacement with new surgery is often required after a few years, a major problem of handicap for old people.

Autologous grafts of bones or cartilage tissue is very difficult and costly. New technologies develop porous matrices implanted as scaffold prosthesis.

These matrices can eventually be filled with growth factors for the tissue regeneration or even with bone marrow stem cells.

However, fixation of cells or factors in the porous matrices with very prolonged and slow release of growth factors is very difficult to achieve and the ideal cocktail and concentration of factors required is unknown.

The aim of the present invention is to provide a homogeneous humanized, bioactive biomaterial (for example porous ceramics) that can be used for implantation purposes and which do not present the long term biocompatibility problems of prior art.

Another aim of the invention is to provide a bioactive biomaterial enabling tissue growth (for example bone and cartilage) in its porous space and securing the integration of the grafted biomaterial in the surrounding tissues (viable bones...).

Another aim of the invention is to provide long lasting prostheses, which avoids requirement for replacement of biomaterial prostheses after 10 years, as often needed up to now.

These aims are achieved by the invention, which consists in humanized biomaterial comprising a porous biocompatible composite material customized and implanted with monocyte derived cells and preferably with macrophages.

The expression "humanized" means that the porous biomaterial has been colonized with human cells derived from blood monocytes.

The expression "biocompatible composite" material designates a material composed of one or several of the following materials proved to be non toxic for human tissues (carbon microfibers, ceramics, calcium phosphates, metal oxides, collagen polymers...).

The expression "porous" means that the biomaterial and preferably the ceramic present pores of about 100 to 2000 microns of diameter.

The expression "customized and implanted" material means that the shape and size of biomaterial is designed specifically for a patient and a site of implantation.

The expression "monocyte derived cells" corresponds to human mononuclear cells isolated from blood, enriched in monocytes and cultured at 37° C in appropriate medium, for 5 to 10 days to obtain tissue type macrophages.

The monocyte derived cells used in the invention are for instance such as those described in PCT/EP 93/01232, WO 99/13054, EP 96/ 901848.0-2107, WO 97/44441.

In a particular embodiment of the invention, the monocyte derived cells described above, contain exogenous compounds such as drugs, proteins, growth factors of interest.

In another embodiment, the monocyte derived cells as described above contain in their cytoplasm exogenous DNA coding for a protein of interest.

The substantially irreversible humanization of matrices of biocompatible composite material described in the present invention allows a physiological interaction between the prostheses made of the biomedical composite, grafted and the host cells in the body. These relations with host tissue cells and with the extracellular matrix allow reconstruction of epithelial sheets and growth of a capillary network around the grafted biomaterial by local multiplication and sprouting of endothelial cells.

The monocyte derived cells, in particular the macrophages used to humanize *in vitro* the porous material in the invention are particularly adequate to increase integration and *in vivo* lifespan of biocompatible prostheses.

Advantageously, the humanized biomaterial of the invention is homogenous.

According to an advantageous embodiment, in the humanized biomaterial of the invention, the biocompatible composite material is chosen among the following materials : microfibres, ceramic materials, metal oxides such as aluminum oxide, calcium phosphate ceramic, glass or carbon fibers, hydroxylapatite, silicon carbide or nitride, collagen polymers or a mixture of these different materials.

According to another advantageous embodiment, in the humanized biomaterial of the invention, the human macrophages are liable to be obtained by *ex vivo* differentiation from blood monocytes leading to living macrophages, and are cultured under conditions enabling their penetration and adherence into the biomaterial for instance for several hours at 37°C, with the porous biomaterial, allowing infiltration of the biomaterial and substantially irreversible binding of the living macrophages to

the biomaterial, now humanized with patient's macrophages and ready for implantation.

The expression "substantially irreversible binding" means that macrophages are tightly bound by numerous contacts with the material and cannot be detached under physiological conditions.

The invention also relates to a living body-supporting implant, characterized by the fact that it comprises or consists of the humanized biomaterial according to any one of claims 1 to 3, and is preferably structured under the form of scaffold, tissue-supporting sponges, bone or cartilage.

The expression "living body-supporting implant" designates an implant having for example the physical form and robustness of a bone to be replaced.

The expression "scaffold" designates a physical structure eventually metallic which keeps the biomaterial (ceramics or other) in the appropriate conformation.

The expression "tissue supporting sponges" designates a soft implant formed for instance of collagen which will be humanized with macrophages before insertion in the body.

The invention also relates to the use of a humanized biomaterial of the invention or of a living body-supporting implant of the invention, for the preparation of a tissue implant destined to replace or repair defective tissue, such as defective bone, cartilage, dental tissue, fibrous tissue, fibrocartilaginous supporting tissue.

The expression "fibrous tissue" designates tissues surrounding organs which support this organ and maintain the shape of this body part : they are mainly formed by epithelial sheets.

According to another advantageous embodiment, the invention relates to the use of a humanized biomaterial of the invention or of a living body-supporting implant of the invention, wherein the monocyte derived cells or macrophages are autologous with respect to the tissue to be replaced or repaired, enabling the biomaterial or the living body-supporting implant to be recognized as self.

The expression "implant to be recognized as self" means that it contains cells (i.e. macrophages) from the patient in which it will be grafted and is therefore autologous to the host.

A process for the preparation of a humanized biomaterial of the invention comprises the following steps :

- preparation of the porous biomaterial structured in form of bones, cartilage,
- preparation of macrophages from blood monocytes,

- immersion of the biomaterial in a physiologic solution appropriate for the culture of macrophages which are added afterwards (ex. : phosphate buffered saline, medium such as RPMI, IMDM, AIMV),
- addition of the macrophage to the solution under conditions enabling binding to the biomaterial and particularly for 1 to 20 h. at 37°C, 5 % CO2 and 5 % air,
- washing of the biomaterial and conservation until use in physiologic medium.

A process for the preparation of a living body-supporting implant of the invention comprises the following steps :

- preparation of a customized porous implant or scaffold composed of bio-compatible material, according to any one of claims 1 to 3,
- preparation of macrophages from blood monocytes of the patient needing the customized implant of biomaterial,
- co-culture of macrophages and the implant in adequate medium under conditions enabling penetration and adherence to the biomaterial, in particular at 37°C, 5% CO2 in hydrophobic bags or containers until grafting the implant.

The invention also relates to the use of the humanized biomaterial or of a living body-supporting implant, which can be implanted in a tissue, for the *in vitro* or *in vivo* or *ex vivo* delivery of factors chosen in the group of chemokines and/or monokines, and/or cytokines and/or growth factors, the factors released being useful for the local attraction of cells required for tissue growth (such as osteoblasts, chondrocytes, fibroblasts, epithelial cells.....) and/or for the neovascularization around the implanted biomaterial, and/or for the release of growth factors sustaining proliferation of cells and/or the growth of new tissues.

Indeed, macrophages maintain tissue homeostasis through the secretion of at least 80 growth factors or monokines controlling and inducing proliferation of mesenchymal (fibroblasts....), endothelial, chondrocytes, osteoblasts, **epithelial** cells.. They also secrete enzymes and mediators allowing growth and renewal of the surrounding cells and tissues (see Table 1).

The key factors secreted by macrophages supporting tissue integration regeneration and growth of mesenchymal cells are : IGF1 and TGFs, but also PDGF, bFGF, MDGF, GM, CSF, NAF, IL-8, TNF, angiogenin and angiogenic factors. These growth factors allow also the development of all the steps required for angiogenesis, allowing neovascularisation and reconstitution of blood microcapillaries around the grafted biomaterial.

In this aspect, macrophages are synthesizing 10 fold more proteins than monocytes, much more growth factors and less inflammatory mediators.

TABLE 1 :

GROWTH FACTORS PROTEINS AND MEDIATORS SUPPORTING TISSUE HOMEOSTASICS SECRETED BY NATURE MACROPHAGES

ENZYMES :

Lyzosymes

Neutral proteases
- Plasminogen activator
- Collagenase
- Elastase
- Angiotensin-convertase

Acid hydrolases
- Proteases
- Lipases
- Ribonucleases
- Phosphatases
- Glycosidases
- Sulphatases

Arginase

COMPLEMENT COMPONENTS
C1, 4, 2, 3 and 5
Factors B and D and Properdin
C1 inhibitor
C3b inactivator and β-1H

ENZYME INHIBITORS
(Antiproteases)

α1-antiprotease
Plasmin inhibitors
α-2 macroglobulin
Plasminogen activator inhibitors

PROTEINS BINDING METABOLIOUS AND LIPIDS :
Acidic isoferritins

BIOACTIVE LIPIDS :

Arachidonic acid metabolites
- Prostaglandins E2, F2α
- Prostacyclin
- Thromboxane
- Leukotrienes B4, C, D and E
- Hydroxy-eicosatetraneoic acids (including SRS-A)

Platelet activating factors

CYTOKINES, HORMONES, GROWTH FACTORS :
- Interleukins 1 α and β
- Tumours necrosis factor α
- Interferons α and β_1, &
- Interleukin 6, 8, 13, 18

Chemotatic factors for
- Neutrophils
- Tlymphocytes
- Monocytes
- Fibroblasts

Heamatopoceitic Colony Stimulating Factors for
- Granulocyte-Macrophages (GM-CSF)
- Granulocytes (G-CSF)
- Macrophages (M-CSF)
- Erythropoeitin

Growth factors
- Fibroblasts growth factor / Insuline like G.F.
- "platgelet-derived growth factor" (PDGF)
- Transforming growth factor α and β
- Endothelial cell growth factor

Hormones
- 1 α, 25-Dihydroxyvitamin D3

Transferrin
Transcobalamin II
Fibronectin
Laminin
Lipid transfer protein
Thrombospondin

NUCLEOSIDES AND METABOLITES :
Thymidine and deoxycytidine
Uracil
Uric acid
Lactic acid
Polyamines nitrines and nitrates
Neopterin

Insulin-like activity, protagandins
Thymosin B4
β endorphin
Adrenocorticotrophic hormone

CHEMOKINES MIP / RANTE FAMILIES
COAGULATION FACTORS :
Factors III, VIII, and tissue factor
Prothrombin and prothrombinase
Factors IX, X, V and VII

In an advantageous embodiment of the invention, the macrophages migrate initially in the porous biomaterial and incorporate irreversibly into this prosthesis by very strong adherence and spreading. When they are kept in physiological conditions, macrophages are very long living cells lasting from several months to several years after implantation. During this time, macrophages will continuously secrete growth factors and cytokines in their local environment ; these factors will act in synergy at very low concentrations (10^{-10} M) on the surrounding cells and tissues.

Furthermore, macrophages do present on their membranes receptors for cytokines, hormones, sugars allowing to respond to micro-environmental needs and to adjust their secretion to the local status around the biomaterial at different periods after grafting.

The growth factors secreted by macrophages represent the global requirements for tissue repair, differentiation and local angiogenesis. The chemokines which will be continuously released in a concentration gradient around the implant will call in and around the prosthesis cells required for recolonization and integration of the biomaterial in host environment. Therefore, the new customized porous biomaterials colonized with host macrophages present a novel biotolerance and a length of adequate performance far better than prosthesis used in the absence of autologous macrophages. Applications are very large in solid or cartilaginous prosthesis needed in bone, cartilaginous repair particularly.

According to another advantageous embodiment, the invention relates to the use of the humanized biomaterial or of a living body-supporting implant, as a graft for the replacement of supporting tissues such as bones, cartilages, dental tissues, epithelial sheet and subcutaneous tissue matrix.

Example 1 :

A calcium phosphate porous ceramic with pores of 200 to 2000 microns (porosity > 20 % and < 80 %) is placed on an hydrophobic support (ethylene vinyl acetate) in the presence of 50 ml AIMV culture medium (life-cell Gibco, Paisley G.B.). Macrophages are added to this preparation at the concentration of 5.10^6 cells/ml ; they are obtained after 7 days differentiation of blood monocytes in culture according to published state of the art in publications and patents (PCT/EP 93/01232, WO 99/13054, EP 96/ 901848.0-2107, WO 97/44441) ; a control preparation is maintained in the absence of macrophages. The preparation is incubated overnight at 37 °C, 5 % CO2, 95 % air to allow fixation of macrophages on the ceramic.

The porous ceramic is washed and cultured in the presence of fibroblasts and/or in the presence of chondrocytes In both cases, the cell proliferation is higher by a factor 2 to 10 for the porous ceramic colonized with macrophages, compared to control ceramic.

Example 2 :

A small fragment of porous microceramic is implanted in a rabbit cornea. The inert microceramic piece induces a very small to moderate inflammation and only peripheral growth of new blood vessels from the ring of the cornea.

In contrast, microceramic covered with macrophages, as described in example 1 induces a major neovascularisation towards the center of the cornea. The cornea becomes vascularized through an invasion of endothelial cells arising from the rim rich in blood supply and sprouting towards the biomaterial implanted.

Example 3 :

Fragments of 100 +/- 20 mg of hydroxyapatite ceramic (Endobon®, Merck) and pieces of one cm2 of a polypropylene scaffold are prepared. Fresh non activated macrophages obtained after 7 days differentiation of blood monocytes in culture according to published patent applications (WO94/26875, WO 99/13054, WO96/22781, WO 97/44441) are suspended (2,5.106 cells/ml) in IMDM (Iscove Modified Dulbecco Medium) culture medium. Each biomaterial fragment is incubated in 1 ml of macrophage suspension, in sterile polypropylene tubes, for 4 hours at room temperature. To check the binding of macrophages on the biomaterial, cells present in the supernatant after incubation are counted. After incubation with Endobon®, from 12 to 17% of the cells added were present in the supernatant (3 experiments),

indicating that more than 2.10^6 macrophages are adsorbed on 100 mg of porous ceramic. After incubation with polypropylene scaffold, from 23 to 55 % of the cells initially added are present in the supernatant, indicating adsorption of 1 to 2.10^6 macrophages/cm2 scaffold.

Nude mice are implanted with biomaterial, and each mouse receives two implants of the same type, one in each flank.

Mice n°	Implanted material
1, 2	Endobon® colonized by macrophages
3	Endobon®
4, 5	Polypropylene colonized by macrophages
6	Polypropylene

Mice are sacrificed after 21 days, macroscopic observation shows no major difference between implants of biomaterial alone and implants of biomaterial colonized by macrophages.

Microscopic observation of tissues in paraffin shows that, when compared to implants of biomaterial alone, implants of biomaterial colonized by macrophages induce first an inflammation phenomenon, which is an important step to induce migration and homing of competent cells for tissue repair. A more important neovascularisation at the implantation site of biomaterial colonized with macrophages has also been observed.

The histological analysis of tissues in resin confirms the increase of neovascularisation for mice implanted with macrophages colonized biomaterials ; when compared to mice implanted only with biomaterial.

The therapeutic applications for tissue repair are confirmed in human bearing non healing ulcers. The ulcers covered with scaffold implanted with autologous macrophages show an improved cicatrization as measured by detersion and size of the ulcer.

CLAIMS

1 - Humanized biomaterial comprising a porous biocompatible composite material customized and implanted with monocyte derived cells and preferably with macrophages.

2 - Humanized biomaterial according to claim 1, wherein the biocompatible composite material is chosen among the following materials : microfibers, ceramic materials, metal oxides such as aluminum oxide, calcium phosphate ceramic, glass or carbon fibers, hydroxylapatite, silicon carbide or nitride, collagen polymers or a mixture of these different materials.

3 - Humanized biomaterial according to claims 1 or 2, wherein the human macrophages are liable to be obtained by *ex vivo* differentiation from blood monocytes leading to living macrophages, and are cultured under conditions enabling their penetration and adherence into the biomaterial, for instance for several hours at 37°C, with the porous biomaterial, allowing infiltration of the biomaterial and substantially irreversible binding of the living macrophages to the biomaterial, being humanized with patient's macrophages and ready for implantation.

4 - Living body-supporting implant, characterized by the fact that it comprises or consists of the humanized biomaterial according to any one of claims 1 to 3, and is preferably structured under the form of scaffold, tissue-supporting sponges, bone or cartilage.

5 - Use of a humanized biomaterial according to any one claims 1 to 3 or of a living body-supporting implant according to claim 4, for the preparation of a tissue implant destined to replace or repair defective tissue, such as defective bone, cartilage, dental tissue, fibrous tissue, fibrocartilaginous supporting tissue.

6 - Use of a humanized biomaterial according to any one of claims 1 to 3 or of a living body-supporting implant according to claim 4, wherein the monocyte derived cells or macrophages are autologous with respect to the tissue to be replaced or repaired, enabling the biomaterial or the living body-supporting implant to be recognized as self.

7 – Process for the preparation of a humanized biomaterial according to any one claims 1 to 3, comprising the following steps :

- preparation of the porous biomaterial structured in form of bones, cartilage,
- preparation of macrophages from blood monocytes,
- immersion of the biomaterial in a physiologic solution appropriate for the culture of macrophages which are added afterwards (ex. : phosphate buffered saline, medium such as RPMI, IMDM, AIMV),
- addition of the macrophages to the solution under conditions enabling binding to biomaterial and particularly for 1 to 20 h. at 37°C,
  5 % CO2 and 5 % air,
- washing of the biomaterial and conservation until use in physiologic medium.

8 – Process for the preparation of a living body-supporting implant according to claim 4, comprising the following steps :

- preparation of a customized porous implant or scaffold composed of bio-compatible material, according to any one of claims 1 to 3,
- preparation of macrophages from blood monocytes of the patient needing the customized implant of biomaterial,
- co-culture of macrophages and the implant in adequate medium under conditions enabling penetration and adherence to the biomaterial in particular at 37°C, 5% CO2 in hydrophobic bags or containers until grafting the implant.

9 – Use of the humanized biomaterial according to any one of claims 1 to 3 or of a living body-supporting implant according to claim 4, which can be implanted in a tissue, for the *in vitro* or *in vivo* or *ex vivo* delivery of factors chosen in the group of chemokines and/or monokines, and/or cytokines and/or growth factors, the factors released being useful for the local attraction of cells required for tissue growth (such as osteoblasts, chondrocytes, fibroblasts, epithelial cells.....) and/or for the neovascularization around the implanted biomaterial, and/or for the release of growth factors sustaining proliferation of cells and/or the growth of new tissues.

10 - Use of the humanized biomaterial according to any one of claims 1 to 3 or of a living body-supporting implant according to claim 4, as a graft for the replacement of supporting tissues such as bones, cartilages, dental tissues, epithelial sheet and subcutaneous tissue matrix.

INTERNATIONAL SEARCH REPORT

Inter[redacted] Application No
PCT/EP 00/08157

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/40 A61L27/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 902 741 A (DUNKELMAN NOUSHIN ET AL) 11 May 1999 (1999-05-11) abstract column 8, line 59 -column 9, line 57 column 10, line 63 -column 11, line 9 column 13, line 12 - line 40 ---	1,2,4-10
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
28 November 2000	05/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Menidjel, R

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	EP 0 798 374 A (MATRIX MEDICAL B V) 1 October 1997 (1997-10-01) column 2, line 6 -column 3, line 31	1,2,4-8

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1,9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten[redacted]pplication No
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